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SEM — Observations on the Shell Plates of Three Polyplacophorans

(Mollusca, Amphineura)

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Abstract

The shell surface of the Polyplacophorans *Chiton olivaceus, Lepidopleurus cajetanus, Acanthochiton fascicularis* was studied by scanning electron microscopy. In the latter two species the aesthete caps terminate at projections of the shell; in *A. fascicularis* these are especially elaborate. The apical caps appear perforated in all three species. There are also pores in the subsidiary caps, but these don't open to shell surface in contrast to the apical caps. The nature of these pores is investigated.

Key-words: Polyplacophora — Shell surface — Aesthete organs — Scanning electron microscopy

Introduction

The outer shell layer of the dorsal valves of the Polyplacophora (the tegmentum) contains a large number of small organs — the aesthetes — which are thought to have some sort of sensory function. Each of these aesthetes consists of a shaft, the megalaesthete, from which small boughs, the micraesthetes, branch off to the shell surface and terminate there with the subsidiary caps. The megalaesthete forms there the apical cap. Normally one aesthete has thirty to sixty cells of various types. Their morphology is known in a few species (Plate 1898/1902, Nowikoff 1907, Knorre 1925), the fine structure has been studied by Boyle (1974) in *Lepidochitona cinereus.* The same author also investigated the shell surfaces of four species of the order Ischnochitonina (1976).

Although there are several studies on the function of these organs (Arey & Crozier 1919, Omelich 1967, Boyle 1972), this question is not quite solved yet;

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Boyle suggests that they are involved in the light response of the animals.[1]) But there remains a lot of problems, e. g. the function of the micraesthetes branching from the main part of an aesthete.

Therefore it is essential to make a more precise study of the structure of the apical and subsidiary caps at the interface to the environment in order to define the range of the possible functions. It is also important to get some information whether or not the caps are uniform among the Polyplacophorans. As the orders are distinguished mainly by their shell structures, modifications in the cap structures could be expected most likely, if they exist, in the comparative study of species from each of these systematical groups.

Material and methods

Specimens of *Chiton olivaceus* (Spengler), *Lepidopleurus cajetanus* (Poli), *Acanthochiton fascicularis (L.)* from the subtidal region at Rovinj / Jugoslavia were kept in a sea water aquarium for about three months. For one part of the investigation some shell plates were treated with a concentrated KOH solution at 90° C; the remaining aragonite structure was cleaned by ultrasonics and dried in air. Other shell pieces were only dried. A third group of plates was decalcified in 6 % EDTA or 5 % HNO_3, embedded in paraplast and cut into sections. After the removal of the paraplast by xylene they were dried. To determine the effects of this treatment the gill microvilli were examined as a control; they seemed to be unaffected. All specimens were coated with gold and studied under a Cambridge stereoscan electron microscope.

Results

The calcareous shell surface varies considerably among the three species. The intermediate valves are always divided into three areas: One median and two lateral fields. First valves generally lack a median field. In *Chiton olivaceus* (Ischnochitonina) the median field exhibits furrows and ribs; in the lateral fields these formations are not so prominent (Fig. 1). All areas of the tegmentum are penetrated by the aesthete canals, but the distribution of them varies in some way. In the lateral fields each megalaesthete opening is accompanied by about nine micraesthete openings. These are arranged in rows which are running in right angle to the lateral border of the plate (Fig. 7). In the median field it is striking how many caps are situated in the furrows; only small aragonite bridges remain between the openings. Here about thirteen micraesthetes branch from one megalaesthete.

In *Lepidopleurus cajetanus* (Lepidopleurina) the median field exhibits regular rows of papillae on the surface (Fig. 3). The aesthetes terminate near the top of the papillae at the side facing the frontal border of the shell. About eight micraesthete openings surround the apical opening (Fig. 8). In the lateral fields the papillae form thick ribs running parallel to the lateral shell border.

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[1]) Meanwhile we have found photoreceptor cells in the aesthetes of all three species (Fischer, F. P., 1978: Photoreceptor cells in chiton aesthetes (Mollusca, Polyplacophora, Chitonidae). — Spixiana 1 (3): 209—213.

In *Acanthochiton fascicularis* (Acanthochitonina) the median and lateral shell parts differ remarkably (Fig. 2). The small median area has a few furrows running parallel the median axis of the animal and a lot of much smaller ones at right angle to it. The distribution of the openings in the median field seems to be quite irregular (Fig. 4); one to seven micraesthetes come on one megalaesthete. The lateral areas of the plates exhibit small elevations which look like pedestals (Fig. 5 and 6). The aesthetes end on the 50—80 μm wide plateaus of these protrusions.

The building of these risings by the lateral epithelium is quite complicated. First a ridge of 25—40 μm length, then at the lateral end of this ridge a cone about 25 μm in diameter are formed (Fig. 6 left). From the top of this cone there runs inside a small tube, which reaches the cone's outside near the upper part of the ridge (Fig. 6, double-arrow). This tube can also be open to the upward side; those pedestals therefore look notched at one side (Fig. 5). The tube is coated with a thin layer of organic material as semithin sections of decalcified valves show. Around the aesthete which is then formed behind the cone by the lateral epithelium calcareous shell substance is built; as a result of this the complete pedestals have an acentric striation (Fig. 5 and 6).

In contrast to the aesthetes of the median tract the lateral aesthetes have only one to three micraesthetes and in many cases none at all in this species. They are also much smaller than the aesthetes in the centre and may consist of nine cells only as compared to about 35. Between the hills the tegmentum is very thin; the aesthete canal may be covered there by an aragonite layer less than one μm thick.

In dried valves which have not been treated with KOH the surface growth of algae and other small organisms which are always found on the shells as well as detritus deserves special attention: A sensory organ which is covered by organisms may be of little use when, for example, it has a function as a photoreceptor.

Fig. 1: *Chiton olivaceus,* intermediate valve of a young animal treated with KOH, 50×

Fig. 2: *Acanthochiton fascicularis,* intermediate valve of a young animal, KOH, 50×

Fig. 3: *Lepidopleurus cajetanus,* last valve of a young animal. Here the two lateral fields form one half-cycle (arrow). KOH, 50×

The lower shell layer, the articulamentum, is not completely covered by the tegmentum.

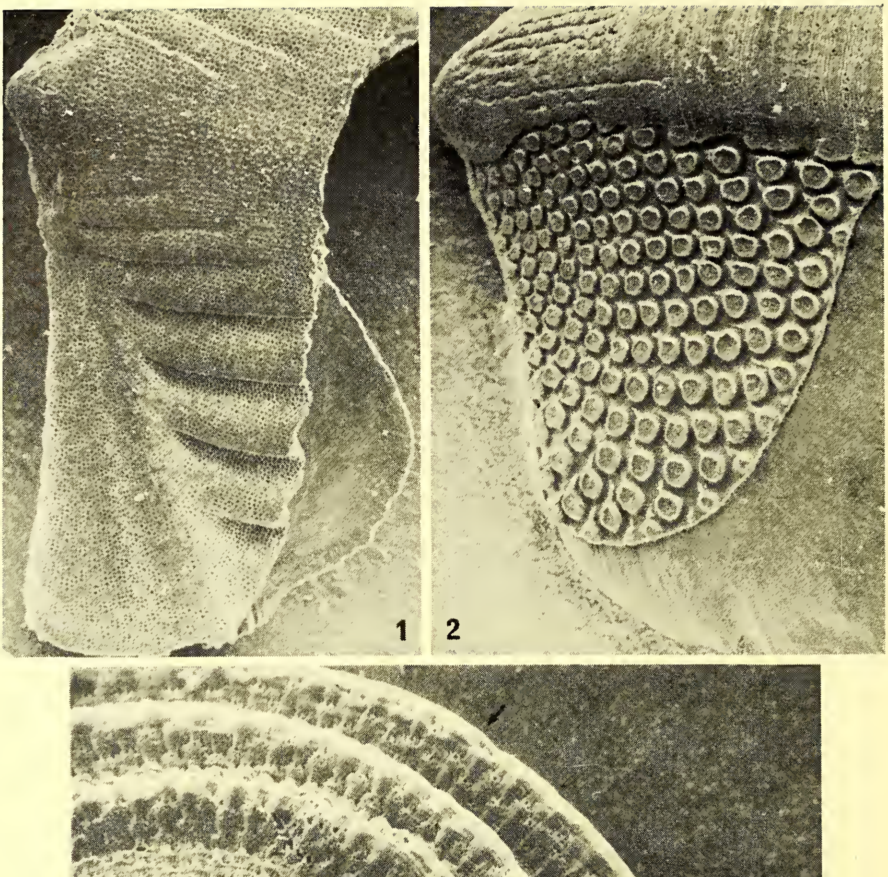
Fig. 4: *Acanthochiton fascicularis,* last valve of a young animal. The median tract ends in the centre of the valve. KOH, 200×

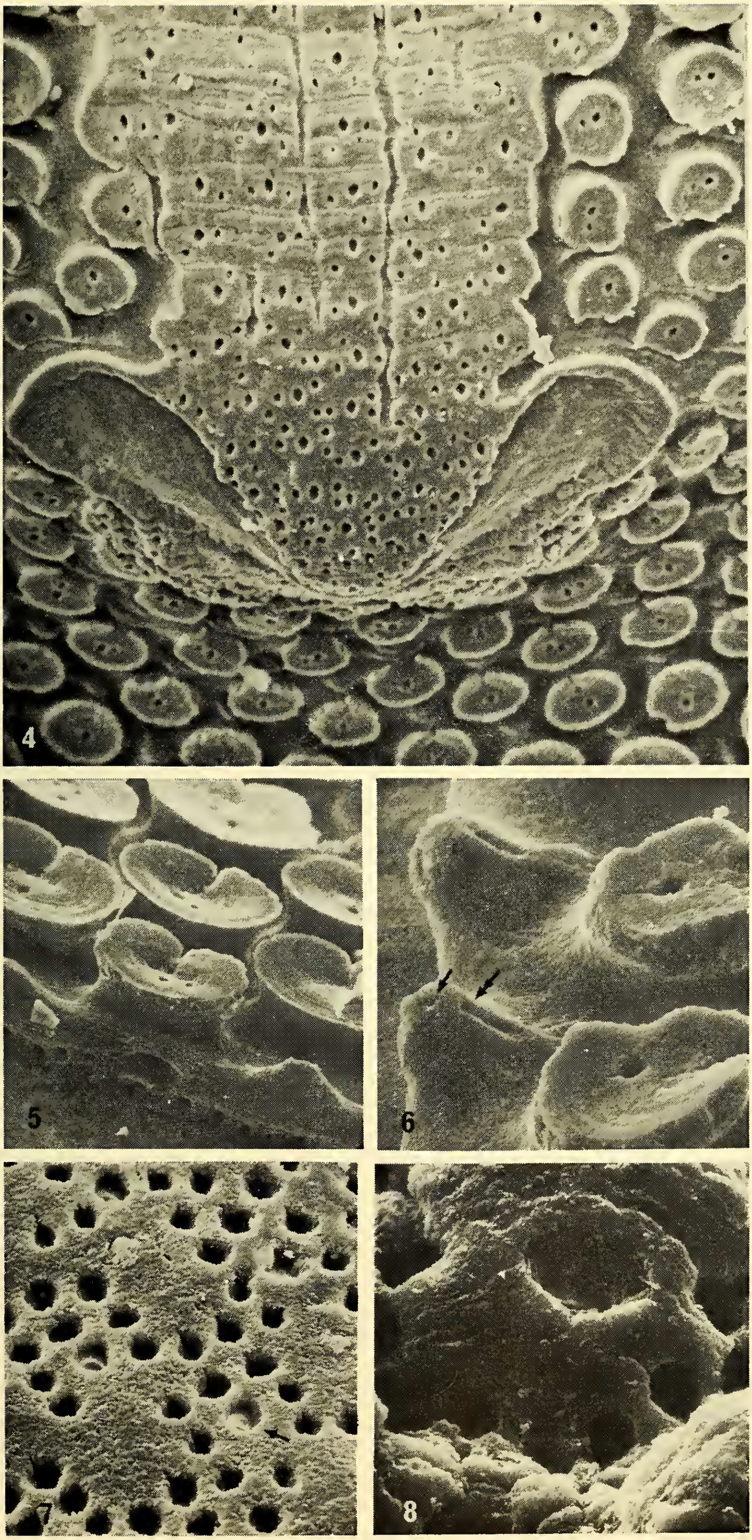
Fig. 5: *Acanthochiton fascicularis,* lateral shell border with hills and aesthete canals passing into the tegmentum. KOH, 250×

Fig. 6: *Acanthochiton fascicularis,* lateral shell border. The hill formation starts with a small ridge and a cone. A small tube (arrow) runs from the cone in the interior to the ridge, which often exhibits a furrow (double-arrow) at the tube's end. The shell-and the aesthetebuilding epithelium has been along the left side. KOH, 250×

Fig. 7: *Chiton olivaceus,* shell surface in the lateral field with apical and slightly smaller subsidiary openings (arrow). KOH, 450×

Fig. 8: *Lepidopleurus cajetanus,* lateral field. The apical pore on top of a papilla is surrounded by smaller subsidiary pores. KOH, 1050×

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Because new shell containing aesthetes is formed continously from the frontal and lateral shell borders there are always intact aesthetes even in old animals in spite of the surf.

In *Chiton olivaceus* the shell surface is relatively clean in most cases. Especially the apical caps are nearly free of contamination even when the surrounding area is encrusted by diatoms.

In *Lepidopleurus cajetanus* and *Acanthochiton fascicularis* the pattern can be understood from the surface structure. In *Lepidopleurus cajetanus* the surface growth converge between the papillae leaving at least the apical caps free. In *Acanthochiton fascicularis* organic material fills the regions between the hills nearly to the edge of them. In these areas many organisms and detritus collect, but the surfaces of the hills remain nearly free (Fig. 11) even when the girdle is covered with silt.

The structure of the aesthete caps seems to be quite uniform in the three species prominating a bit over the surrounding shell surface in a slightly convex pattern. Their diameters vary to some extent: In *Chiton olivaceus* the apical caps measure 16—19 μm, the subsidiary caps 9—14 μm (even within one group of caps), in *Lepidopleurus cajetanus* 13—15 μm and about 9 μm, in *Acanthochiton fascicularis* 8—11 and 5—9 μm respectively.

In all three species the apical caps are perforated by small pores of a diameter of 40—200 μm which are seperated from each other by 40—100 μm thick bridges. In *Lepidopleurus cajetanus* and in *Acanthochiton fascicularis* the pores are not quite as regular as in *Chiton olivaceus.* Often these pores can be seen open to the shell surface (Fig. 10). In younger shell parts a thin organic layer, the periostracum, covers the surface including the caps with the pores. Frequently this layer is seen to disappear first from the apical caps, beginning at the areas immediately above the pores (Fig. 15). It is unlikely that this process is due only to surf action; similarly exposed areas such as the tops of the shell papillae in *Lepidopleurus cajetanus* lose their periostracum much later. Sometimes on those caps where the layer starts to disappear piles of matter can be seen, which have about the same diameter as the pores (Fig. 15, arrow). The pores of the apical caps are often filled with organic material in young aesthetes as TEM studies show. In some of our animals, especially in *Acanthochiton fascicularis,* the apical caps are covered with a very thin sheet even in shell areas where the periostracum has disappeared.

It is essential in the study of the aesthete organs to determine whether these pores pass through the cap to the interior of the aesthete or not. At the edges of broken shell pieces which have not been decalcified, as well as in decalcified sections, the pores penetrate the cap to its proximal surface (Fig. 13 and 14). Here the cap is covered by a layer of organic material which doesn't show special pores. But according to TEM-observations this layer is not continous; it has small openings of a diameter of around 50 nm.

There are no areas in the caps which lack pores. In cases in which this could be suggested from shell surface observations remaining periostracum may be the reason. There is no difference in the appearance of decalcified and undecalcified caps. The pores appear not to be filled with any matter in elder aesthetes but there remains the possibility that they normally contain a volatile substance which has been lost during drying and evacuation of the specimens.

Breaches of dried shells and decalcified sections also reveal details about the structure of the subsidiary caps. In all three species they contain pores.[2]) But these never open to the surface; they are closed to the interior of the micraesthete, too (Fig. 12 and 16). In these pores no material could be found. No calcareous parts, neither in the apical nor in the subsidiary caps, could be detected.

Discussion

The structure of the shell surface and the position of the aesthete caps show marked differences among the investigated species. In *Lepidopleurus cajetanus* and especially in *Acanthochiton fascicularis* the endings of the aesthetes and the pattern of the surface structure are strictly correlated. In these species, which don't possess as many aesthete endings as *Chiton olivaceus*, the surface pattern involves an effective exposure of the caps to the environment.

The connection of some aesthete cells with the lateral nerve (Knorre 1925, Omelich 1967), their fine structure (Boyle 1974) and the results of behavioural studies (Arey & Crozier 1919, Boyle 1972) suggest that the aesthetes are sensory organs. It is crucial in the investigation of their exact function to determine the structure of the caps because they may serve as filters of external stimuli. Omelich (1967) and Boyle (1974, 1976) showed by transmission electron microscopy that the apical caps appear perforated. Haas (1972) described an aragonite framework in the apical cap of *Chiton marmoratus*, and Boyle (1976) interprets the pores in the TEM-pictures as areas where this framework has been removed by decalcification. He considers also that the porous appearance of the apical caps in dried shells of *Sypharochiton pelliserpentis* seen in SEM-photos is due to a loss of the organic matrix of the cap.

Fig. 9: *Chiton olivaceus,* untreated shell surface with one apical and a few subsidiary caps. 2000×

Fig. 10: *Chiton olivaceus,* apical cap with pores. The periostracum has not yet disappeared from the surrounding area. 4900×

Fig. 11: *Acanthochiton fascicularis,* untreated shell surface. The pedestals' tops are nearly clean. 550×

Fig. 12: *Chiton olivaceus,* section (decalcified) showing an apical cap and a cut subsidiary cap. Pores are seen inside the subsidiary cap. 5000×

Fig. 13: *Chiton olivaceus,* untreated shell. Breach through the apical cap. 4500×

Fig. 14: *Chiton olivaceus,* section (decalcified) through an apical cap. 5000×

Fig. 15: *Acanthochiton fascicularis,* apical cap near the lateral shell border. The periostracum starts to disappear (right side of the Fig.) and piles of matter (arrow) can be seen at the left side, where the periostracum has disappeared to some extent. Decalcified, 5000×

Fig. 16: *Chiton olivaceus,* untreated shell. At this breach ridge a micraesthete cell body can be seen under the subsidiary cap. 4500×

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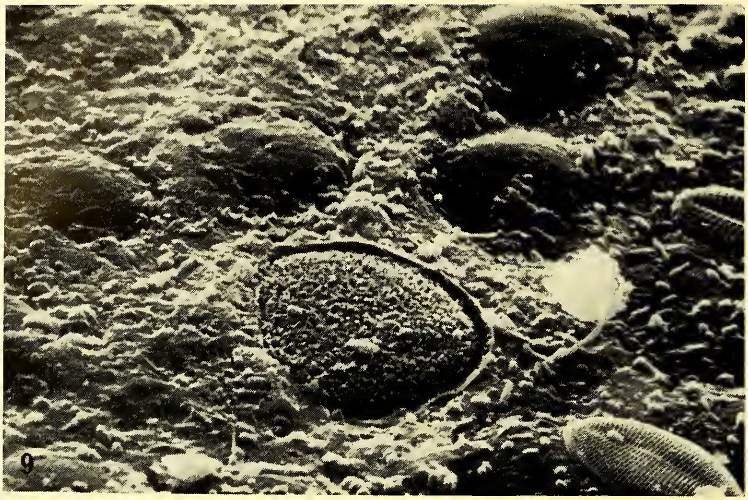
[2]) Only the very small lateral aesthetes of *Acanthochiton fascicularis* lack those pores.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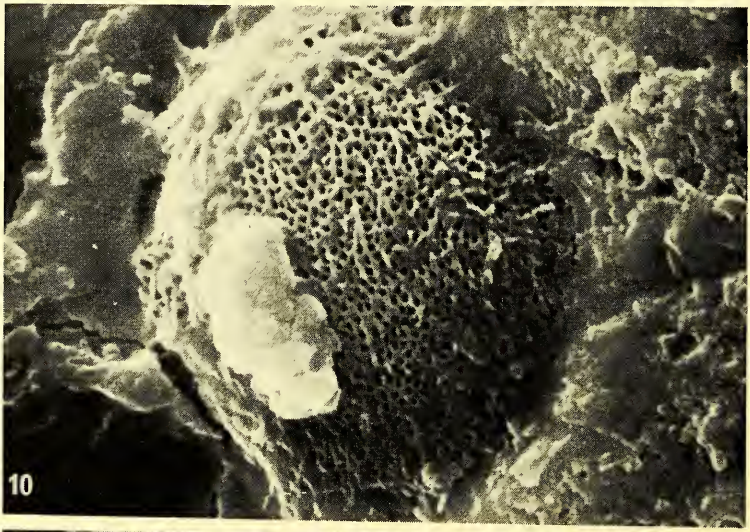
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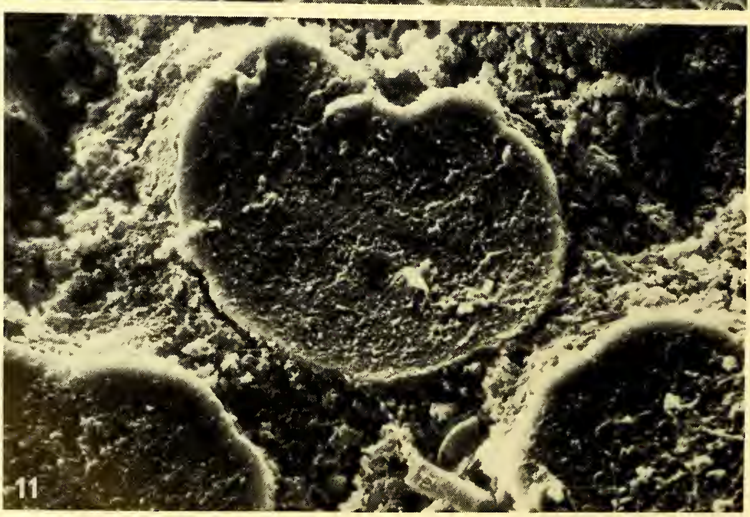
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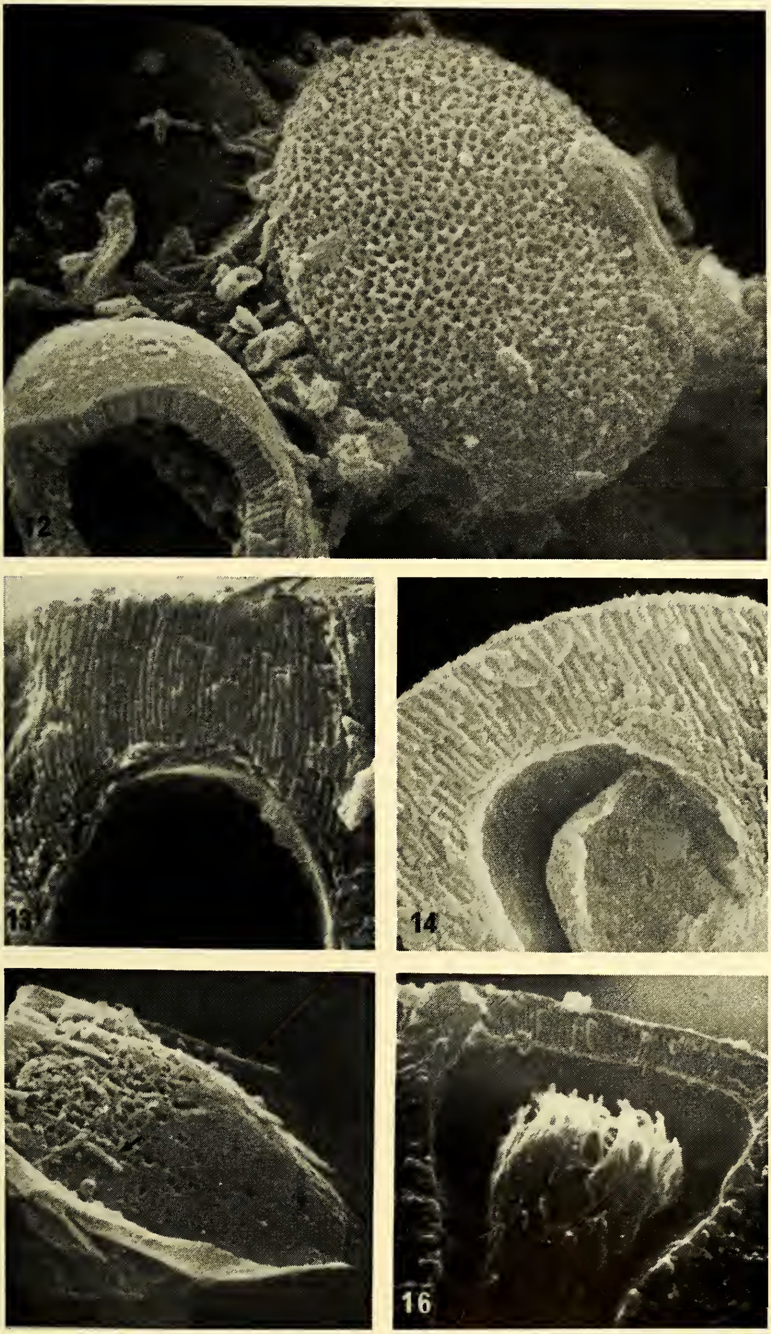
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In our investigations no calcareous framework could be found. The fact that dried caps at breach ridges as well as decalcified sections give the same appearance in SEM-photos with canals passing to the proximal region of the cap suggest that these pores are the normal feature of the apical cap. There may remain some possibility of an interaction of environmental factors to the aesthete sensory structures. The fact that intact aesthetes may have distally open cap pores or not suggests that either these are two different functional states or that open pores are not necessary for their main function.

The pores of the subsidiary caps don't open to the surface in any of the examined shell plates. Boyle (1976) found similar pores in *Onithochiton neglectus* by transmission electron microscopy. But according to his observations in *Lepidochitona cinereus* (1974) it seems that this species has no pores in its subsidiary caps. Therefore it is not clear whether the porous appearance of the subsidiary caps of all other investigated species is a normal feature of the micraesthetes and possibly of importance for their function.

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